DIETARY ELECTROLYTES AND THEIR INFLUENCES ON PLASMA ALDOSTERONE CONCENTRATION

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DEDICATION

To my beloved parents,

You gave me strength when I thought of giving up. You provide your moral, spiritual, emotional, and financial support. You give me free to do everything I want to do. I am so lucky that I have such wonderful family.

To my aunt Juan,

You help me apply for this major and give me a lot of help when I needed. Without you, I would not have had such a precious learning experience.

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DIETARY ELECTROLYTES AND THEIR INFLUENCES ON PLASMA ALDOSTERONE CONCENTRATION

Excessive sodium retention is thought to be the main culprit for hypertension. The modern American diet provides an excess of sodium and not sufficient amount of sodium. In this research, we examined the relations among urinary sodium and potassium excretion, and plasma levels of aldosterone, a mineralocorticoid hormone that has been linked to incidence hypertension, in a cohort of healthy children and young adults. We found that higher plasma aldosterone level was associated with lower sodium excretion in the urine, in blacks and whites, suggesting that aldosterone plays a critical role in retaining the sodium from dietary sources. The study highlights the importance of dietary sodium restriction.

Wanzhu Tu, Ph.D., Chair

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LIST OF ABBREVIATIONS

BMI: Body Mass Index

BP: Blood Pressure

ENaC: Epithelial Sodium Channel

K: Potassium

Na: Sodium

PAC: Plasma Aldosterone Concentration

Background

The risk of hypertension increases with age, although the exact mechanisms of age-related blood pressure (BP) elevation have not been fully elucidated (Whelton et al., 1994). Excessive sodium retention is thought to be the main culprit of hypertension. Aldosterone, a potent mineralocorticoid hormone, is an important participant to sodium retention in the kidney, through the epithelial sodium channel (ENaC) in the collecting duct. Studies have shown that excessive secretion of aldosterone causes BP to increase (Vasan et al., 2004). However, plasma aldosterone concentration (PAC) decreases with age, while incidence of hypertension increases.

Recent studies have found that in blacks, BP could increase at a modest level of plasma aldosterone concentration, a phenomenon herein referred to as aldosterone sensitivity (Tu et al., 2014). Compared to whites, aldosterone sensitivity in blacks increases with age and blacks has a higher salt sensitivity (blood pressure rises with increasing sodium intake). At the same time, the average PAC level was lower in blacks, as compared to whites (Tu et al., 2018).

Another factor contributing to the higher incidence and prevalence of hypertension is the imbalance in dietary sodium and potassium intake (Adrogue et al., 2007). Daily consumption of high-salt foods can lead to an increase in sodium in the body. Insufficient vegetable and fruit consumption often lead to potassium deficiency. But Previous studies have not explored the effects of dietary sodium and potassium on aldosterone.

In the present study, we comparatively analyzed sodium and potassium intake, as approximated by their respective urine excretion rates, and their influences on

aldosterone production in healthy blacks and whites, by using the longitudinal data generated by an observational study. The findings could have implication to the diet effects of sodium and potassium on the development of hypertension.

Method

Subjects and Study Design

We analyzed data from an observational study that had begun in 1986 (the Child Study) (Tu et al., 2011). The study subjects were healthy children including blacks and whites attending schools in Indianapolis, Indiana. Children between the ages from 5 to 17 were recruited for participation, and BP of the enrolled subjects was measured twice a year. The study also collected overnight urine samples. Most of the study participants also provided blood samples, in at least one occasion. Individuals with a history of cardiovascular or renal disease, hypertension, diabetes, and medications that take blood pressure changes were excluded from participating in the study.

Second follow-up (the Adult Study) began in 2008 and ended in 2013, using the original study protocol except for blood samples were collected twice a year (Tu et al., 2017). The study again excluded subjects with a history of treatment for hypertension. An Internal Review Board at Indiana University approved the research program. The study received consent from children, parents of children and adults.

Measurements

BP in the Child Study was measured every six months, by using a random zero sphygmomanometer (Hawksley & Sons, Lancing, West Sussex, United Kingdom). BP in the Adult Study was measured by a mercury Baumanometer Standby Model (W.A. Baum Company, Copiague, New York). We measured BP in the right arm after the subject sat down for 5 minutes. The first and fifth Korotkoff sounds were used to determine the systolic and diastolic BP. Three BP readings were recorded for each test. The average of the last two values was used in the current analysis. There were no dietary requirements

for children and adults. The age was calculated as the length of time from the date of birth to BP measurement. The race categories were determined by self-report. The body mass index (BMI) was calculated from the recorded height and weight when they were assessed.

Sample Processing and Assay Procedures

The blood sample was stored in a frozen environment at minus 80 degrees Celsius after treatment at room temperature. Analysis of blood samples was completed within three weeks of collection and thawed during analysis. Urine sample used overnight urine. The measurement of aldosterone required hydrolysis. Radioimmunoassay with antiserum from Diagnostic Products Corporation (Los Angeles, CA) was used to measure aldosterone concentrations in plasma and urine. The concentrations of sodium and potassium in plasma and urine were measured by flame photometry. The excretion rates of potassium and sodium in the urine were used to estimate the intake of sodium and potassium.

Statistical Analysis

The demographic and clinical characteristics were presented in tabular form, including baseline demographic characteristics and plasma and urine measurements. Baseline characteristics of the study participants were compared between blacks and whites using the t-test for continuous variables and chi-square test for discrete random variables.

Semiparametric regression models were used to describes nonlinear relationships as regression splines to depict the age-related changes in the concentrations of sodium

and potassium in the plasma and urine. Estimated mean the concentrations of sodium and potassium in plasma and urine at different ages fitting results were smooth curves of age.

Bivariate semiparametric regression analyses were performed to examine the simultaneous effects of sodium and potassium intake, as approximated by urine measurements, on aldosterone levels (Ruppert et al., 2004). Estimated effects were presented as colored contour plots. All analysis was performed using R software (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org/). Statistically significant were considered when P-value smaller than 0.05.

Results

The data we used for the current analysis were contributed by 394 child participants and 232 adult participants. Baseline characteristics of participants to the Child Study and the Adult Study were described in Table 1.

Summary statistics of plasma and urinary measurements averaged over age in children and adults were provided in table 2. PAC was lower in blacks than in whites at any age (P-value < 0.0001). The average levels were 9.07 ng/dL for black children and 14.07 ng/dL in white children. Comparing to children, adults had a lower level of PAC, averaging 5.09 ng/dL and 9.26 ng/dL for blacks and whites, respectively. Urine aldosterone levels were similarly lower in blacks.

Table 1

Baseline Characteristics of Study Subjects When They Were Children and Then Again When Some of the Same Subjects Were Adults

Variables	Total	Blacks	Whites	P-value
Characteristics of the Child Study participants at the study entry				
N	394	146	248	
Male	204	71	133	
Age, y	14.34 (1.82)	13.97(1.80)	14.52(1.80)	0.0009
Systolic BP, mm Hg	108.65 (11.74)	108.77 (11.70)	108.60(11.78)	0.8768
Diastolic BP, mm Hg	65.97(10.22)	67.63(9.68)	65.14(10.40)	0.0100
BMI, kg/m	22.42(4.85)	23.93(5.82)	21.66(4.09)	<0.0001
Characteristics of the Adult Study participants at the study entry				

N	232	89	143	
Male	112	41	71	
Age, y	30.99 (3.97)	30.19(3.97)	31.53(3.87)	<0.0001
Systolic BP, mm Hg	115.66(11.87)	116.95(12.21)	114.78(11.57)	0.3683
Diastolic BP, mm Hg	73.48(10.20)	74.57(10.93)	72.74(9.62)	0.0387
BMI, kg/m	29.16(7.73)	31.77(7.77)	27.38(7.19)	0.0258
Note: BMI indicates body mass index; and BP, blood pressure.				

Table 2

Summary Statistics of Plasma and Urinary Measurements Averaged Over Age in Children and Adults

	Total	Blacks	Whites	P-value
Biochemical measures from the Child study				
Plasma K, mmol/L	4.24(0.50)	4.37(0.73)	4.20 (0.37)	0.0305
Plasma Na, mmol/L	138.51(3.37)	138.01(3.07)	138.70(3.45)	0.0801
Urine K, mmol/g Cr*	24.46(16.20)	23.02(13.01)	25.18(17.56)	0.1300
Urine Na, mmol/g Cr*	96.11(54.46)	91.84(46.34)	98.25(58.06)	0.1914
PAC, ng/dL*	12.40(8.75)	9.07(6.61)	14.07(9.21)	<0.0001
Urine aldosterone, μg/mg Cr*	4.59(4.64)	3.71(4.38)	5.03(4.71)	0.0024

Biochemical measures from the Adult study				
Plasma K, mmol/L	3.81(0.28)	3.80(0.27)	3.01(0.28)	0.5830
Plasma Na, mmol/L	138.46(2.12)	138.29(2.32)	138.58(1.97)	0.0900
Urine K, mmol/g Cr*	19.95(12.83)	17.99(8.56)	21.29(14.93)	0.0003
Urine Na, mmol/g Cr*	79.69 (44.88)	76.92(40.83)	81.58(47.40)	0.1745
PAC, ng/dL*	7.78(8.05)	5.59(4.91)	9.26(9.34)	<0.0001
Urine aldosterone, μg/mg Cr*	4.66(4.97)	3.99(1.50)	5.11(5.60)	<0.0001
Note: PAC indicates plasma aldosterone concentration; *P values were obtained from 2 sample t tests based on logarithmic transformed data.				

The estimated urinary sodium and potassium excretion rates over age were presented in Figure 1 and 2. Both slightly decreased with age. Similar time trends were observed in plasma potassium and sodium concentrations observed in study participants in Figure 3 and 4.

Bivariate semiparametric regression analysis showed that (1) urine sodium and potassium levels are correlated with PAC (see Figure 5). Figure 5 showed that aldosterone promotes potassium disposal and sodium retention: When PAC is higher, potassium excretion is higher and sodium excretion is lower.

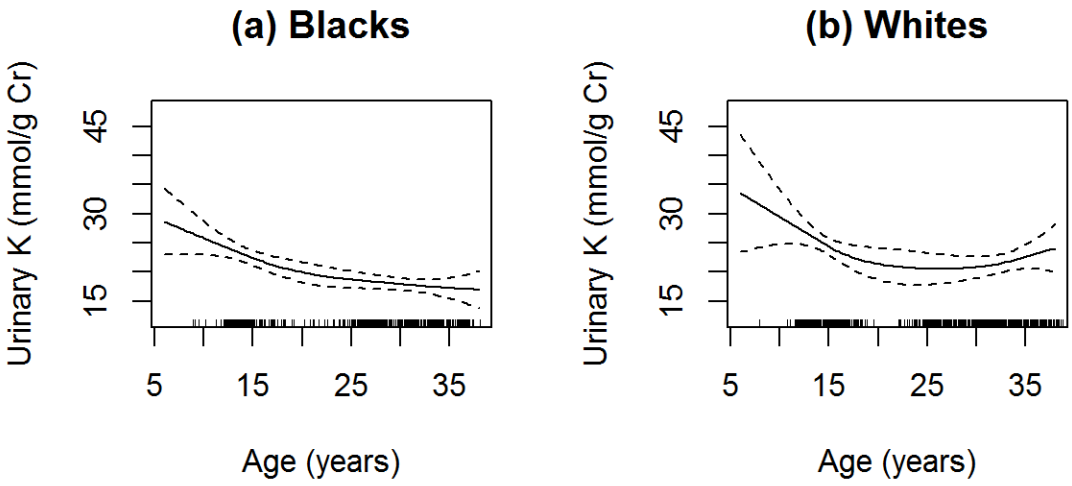

Figure 1: Age-related changes in urine potassium

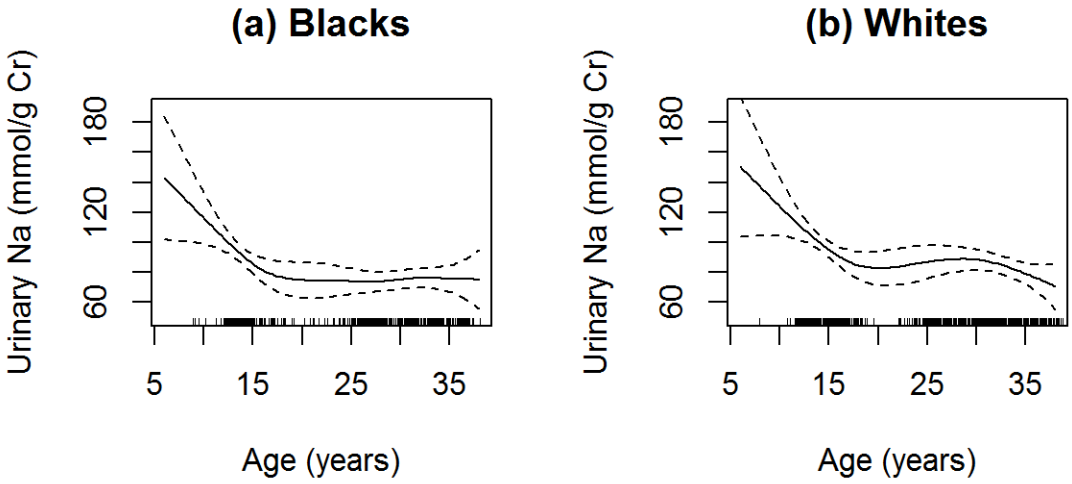

Figure 2: Age-related changes in urine sodium

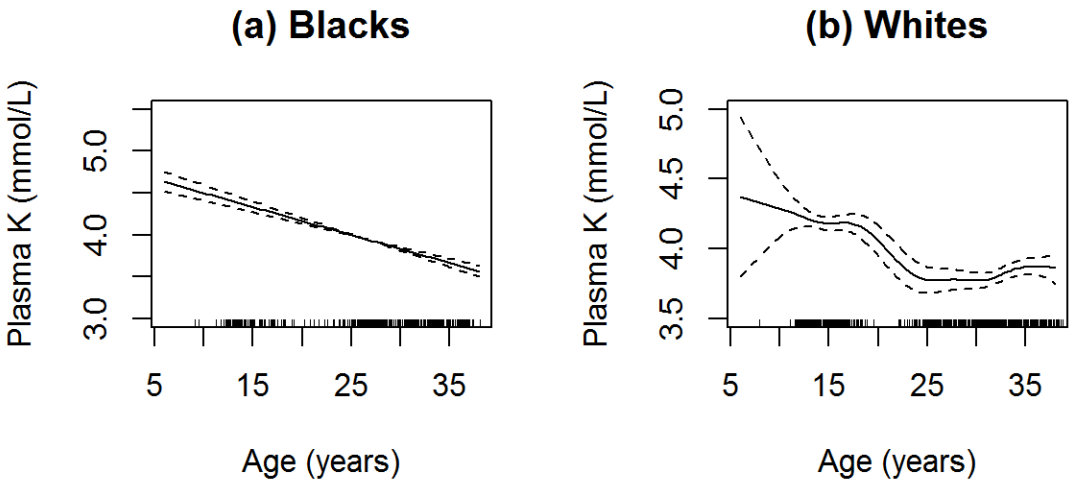

Figure 3: Age-related changes in plasma potassium concentration

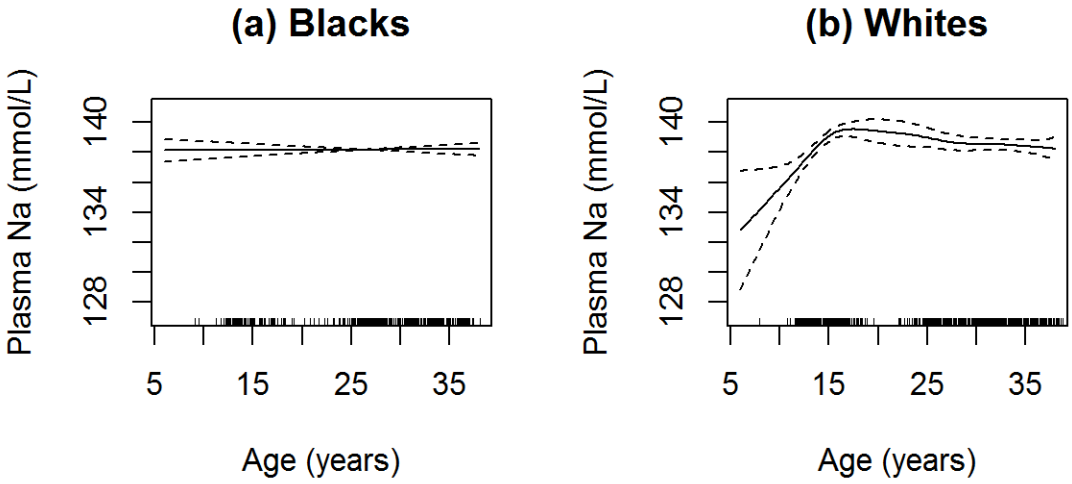

Figure 4: Age-related changes in plasma sodium concentration

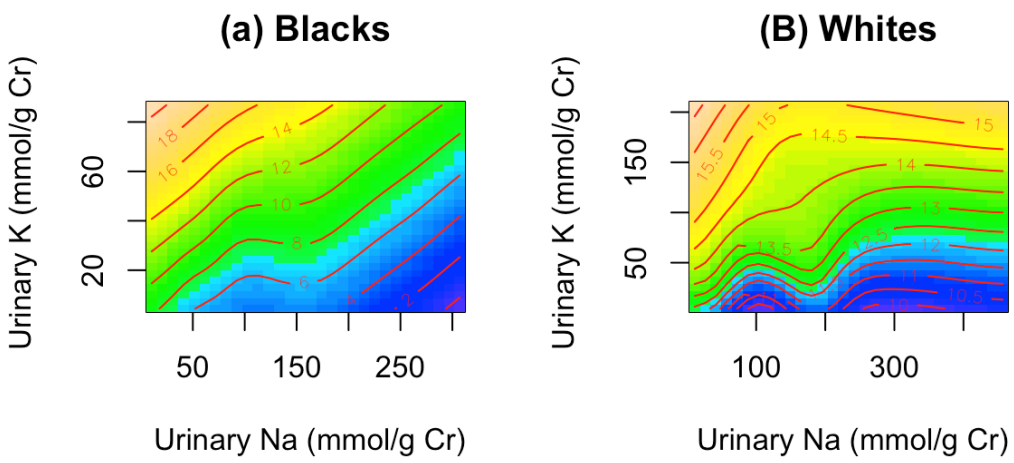

Figure 5: Plasma aldosterone concentration in blacks and whites

Discussion

As a mineralocorticoid hormone, aldosterone plays a crucial role in the pathophysiology of hypertension. Although aldosterone levels in the circulation are lower in blacks, the difference did not automatically translate to lower blood pressure. Higher aldosterone levels are associated with lower sodium excretion in the urine, in blacks and whites, suggesting that aldosterone plays a critical role in retaining the sodium from dietary sources. The study highlights the importance of dietary sodium restriction.

References

Adrogué, H. J., & Madias, N. E. (2007). Sodium and potassium in the pathogenesis of hypertension. *New England journal of medicine*, *356*(19), 1966-1978.

Ruppert, D., Wand, M. P., & Carroll, R. J. (2003). *Semiparametric regression* (No. 12). Cambridge university press.

Tu, W., Eckert, G. J., DiMeglio, L. A., Yu, Z., Jung, J., & Pratt, J. H. (2011). Intensified effect of adiposity on blood pressure in overweight and obese children. *Hypertension*, *58*(5), 818-824.

Tu, W., Eckert, G. J., Hannon, T. S., Liu, H., Pratt, L. M., Wagner, M. A., ... & Pratt, J. H. (2014). Racial differences in sensitivity of blood pressure to aldosterone. *Hypertension*, *63*(6), 1212-1218.

Tu, W., Eckert, G. J., Decker, B. S., & Howard Pratt, J. (2017). Varying influences of aldosterone on the plasma potassium concentration in blacks and whites. *American journal of hypertension*, *30*(5), 490-494.

Tu, W., Li, R., Bhalla, V., Eckert, G. J., & Pratt, J. H. (2018). Age-related blood pressure sensitivity to aldosterone in blacks and whites. *Hypertension*, *72*(1), 247-252.

Vasan, R. S., Evans, J. C., Larson, M. G., Wilson, P. W., Meigs, J. B., Rifai, N., ... & Levy, D. (2004). Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *New England Journal of Medicine*, *351*(1), 33-41.

Whelton, P. K. (1994). Epidemiology of hypertension. *Lancet (London, England)*, *344*(8915), 101-106.

Curriculum Vitae

Xiaohan Ye

Education

Indiana University–Purdue University — Indianapolis, Indiana, USA
Master of Science in Biostatistics — Aug. 2017-June 2019

Inner Mongolia University — Inner Mongolia, P.R. China
Bachelor of Science in Information and Computing Science — Sept. 2012-July 2016

Experience

***Data Analyst,* Indiana University, School of Medicine**
Sept. 2018-Present
Project: Age effect on blood pressure development; Performed semiparametric analysis on metabolites and hormones regulating blood pressure

- Self-learning R language and programming all analysis; Self-learning semi-parametric analysis for analyzing programming results; Writing a draft of the thesis.

***Math Teacher,* The 8th Middle School**, Wuhai City, Inner Mongolia, P.R. China
Sept.-Oct. 2016

- Taught seventh grade mathematics; Delivered daily instruction to 52 students ages 11-12; managed Math Team class activities.

***Team leader,* Inner Mongolia University**, School of Mathematical Sciences
Apr. 2014-May 2016
Project: Electroosmotic flow of third grade fluid between microparallel plates with magnetic flied

- Formed a team and applied to a national foundation of college innovation; Completed manual calculation of the equation and wrote the paper; Planned team-learning activities and cost reimbursement.

Awards

The Third Place of Outstanding Graduates Awards — 2016
The Second Place Scholarship — 2015
The Third Place Scholarship — 2014